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Identification of Thermophilic Bacteria in Solid-Waste Composting†

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The thermophilic microbiota of solid-waste composting, with major emphasis on *Bacillus* spp., was examined with Trypticase soy broth (BBL Microbiology Systems) with 2% agar as the initial plating medium. Five 4.5-liter laboratory units at 49 to 69°C were fed a mixture of dried table scraps and shredded newspaper. The composting plants treating refuse at Altoona, Pa., and refuse-sludge at Leicester, England, were also sampled. Of 652 randomly picked colonies, 87% were identified as *Bacillus* spp. Other isolates included two genera of unidentified nonsporeforming bacteria (one of gram-negative small rods and the other of gram-variable coccobacilli), the actinomycetes *Streptomyces* spp. and *Thermoactinomyces* sp., and the fungus *Aspergillus fumigatus*. Among the *Bacillus* isolates, the following, in order of decreasing frequency, were observed: *B. circulans* complex, *B. stearothermophilus*, *B. coagulans* types A and B, *B. licheniformis*, *B. brevis*, *B. sphaericus*, *Bacillus* spp. types i and ii, and *B. subtilis*. About 15% of the *Bacillus* isolates could be assigned to species only by allowing for greater variability in one or more characteristics than has been reported by other authors for their strains. In particular, growth at higher temperatures than previously reported was found for strains of several species. A small number of *Bacillus* isolates (less than 2%) could not be assigned to any recognized species.

A large variety of thermophilic microorganisms have been reported in composting and other self-heating organic materials (Table 1). However, systematic attempts to identify the thermophilic bacteria other than actinomycetes are lacking. Such information is of particular interest because these bacteria may be the major active organisms in the thermophilic stages of composting.

As part of a study to determine the effect of temperature on species diversity in solid-waste composting (72; P. F. Strom, Ph.D. thesis, Rutgers University, New Brunswick, N.J., 1978), bacteria isolated from laboratory and field composting samples at various temperatures were identified. Emphasis was placed on the genus *Bacillus* because of indications that it might be the major component of the thermophilic microbial community (26, 68; M. L. Morris, M.S. thesis, Rutgers University, New Brunswick, N.J., 1975).

MATERIALS AND METHODS

Laboratory composting was performed in a 4.5-liter reactor placed in an incubator for temperature control. A mixture of table scraps and shredded newspaper was used as feed, and samples were collected from five runs at temperatures ranging from 49 to 69°C. Samples were also collected from the Fairfield-Hardy composting unit at Altoona, Pa., and from two replicate Dano drums and two curing windrows of different ages at Leicester, England. There were thus a total of nine samples, not counting replicates. Details are given elsewhere (72; Strom, Ph.D. thesis).

Appropriate dilutions of each sample were surface plated on Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 2% agar (TSA). Incubation was at the composting temperature for laboratory composting samples, at 61 to 62°C for Altoona samples, and at 48 to 49°C for Leicester samples. After 20 h of incubation, a number of colonies were picked randomly (all colonies on a plate or sector) and streaked onto fresh TSA plates. Additional colonies of unusual appearance were picked deliberately to try to increase the number of different species isolated. After incubating the original spread plates for another 24 h, a few additional deliberately selected colonies were streaked.

Cultures which grew on the TSA streak plates were transferred to soil extract agar slants (35). If a culture did not grow, an attempt was made to restreak it from the original spread plate. Occasionally, more than one colony type appeared on the streak plates; in such cases, each type was restreaked until pure cultures of all were obtained. Cultures were stored on soil extract agar slants at 4°C. Leicester cultures were sent to the United States on soil extract agar slants transported at ambient temperatures for 1 week.

Isolates which macroscopically appeared to be actinomycetes or fungi were streaked onto plates of 10% normal-strength Trypticase soy broth with 1.5% agar and observed microscopically. Assignment to genus or species was based on temperature tolerance limits and filament and spore morphology as in standard references for actinomycetes (6, 17) and fungi (13, 61).

Nonfilamentous cultures were tentatively considered members of the genus *Bacillus* and subjected to an identification scheme by the methods of Gordon et al. (35). Stock cultures were included in each series of tests as positive and negative controls.

Nonsporeforming bacteria were additionally stained for Gram reaction and in some cases for flagella (69), and two cultures were tested with API 20 *Enterobacteriaceae* diagnostic strips (Analytab Products, Inc., Plainview, N.Y.). Skerman's key (66) was then consulted.

RESULTS

Of the 652 randomly picked colonies, 567 (87%), comprising nine taxa, were identified as members of the genus *Bacillus*. Including the deliberately picked isolates, 10 taxa of *Bacillus* were represented. Table 2 shows the number of samples in which each taxon was present, the number of isolates, and a summary of identification test results.

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TABLE 1. Microorganisms reported in self-heating materials at thermophilic temperatures[a]

Classification and species[b]	Reference
Fungi	
Zygomycetes	
Absidia ramosa	11, 18
Absidia sp.	25, 40
Mortierella turficola	41
Mucor (*Absidia*) *corymbifera* (*A. lichtheimi*)	53, 79
M. miehei	14, 25, 47
M. pusillus	11, 14, 18, 22, 25, 40, 41, 44, 47, 57, 79
Rhizomucor sp.	57
Ascomycetes	
Allescheria terrestris	18
Byssochlamys sp.	14
Chaetomium thermophilum	10, 11, 14, 18, 22, 44, 47, 54, 75, 79
Dactylomyces crustaceous	47
Myriococcum albomyces	14
Talaromyces (*Penicillium*) *dupontii*	11, 14, 18, 22, 79
T. (*P.*) *emersonii*	75
T. (*P.*) *thermophilus*	18, 54, 75
Thermoascus aurantiacus	14, 18, 44, 53, 54, 75, 79
Thielavia (*Sporotrichum*) *thermophila*	24, 42
Basidiomycetes	
Coprinus lagopus	14
Coprinus sp.	11, 14
Lenzites sp.	18
Deuteromycetes	
Aspergillus fumigatus	9–12, 14, 18, 22, 25, 40, 41, 43, 44, 47, 53, 54, 56, 74, 75, 79
A. niger	53
Humicola grisea var. *thermoidea*	14, 22, 41, 54, 75
H. insolens	11, 14, 18, 22, 41, 43 (14), 81 (14)
H. (*Thermomyces*) *lanuginosa*	10, 11, 14, 18, 22, 25, 40, 41, 44, 47, 53, 54, 56, 57, 75, 79
H. stellata	14, 43, 75
Malbranchea pulchella var. *sulfurea* (*Thermoidium sulfureum*)	11, 14, 44, 53, 79
Scytalidium thermophilum	18
Sporotrichum thermophile	11, 18, 42, 43, 75
Stilbella thermophila	22, 41
Torula thermophila	14, 41, 47, 54
Mycelia Sterilia	
Papulaspora thermophila	23
Unidentified	11, 18, 79
Actinomycetes	
Actinobifida chromogena (*Thermomonospora fusca*)	22, 51, 81
Microbispora (*Thermopolyspora*) *bispora*	43
Micropolyspora faeni (*Thermopolyspora polyspora*)	22, 25, 40, 51
Nocardia brasiliensis[c]	22
Nocardia sp.	51
Pseudonocardia thermophilia	22, 43
Streptomyces rectus[d]	22, 41, 43, 71
S. (*Actinomyces*) *thermofuscus*[d]	81
S. (*A.*) *thermophilus*[d]	44, 53, 81
S. thermoviolaceus	22, 71
S. thermovulgaris	22, 41, 71
S. violaceus-ruber (*violaceoruber*)	22
Streptomyces (*Actinomyces*) sp.	51, 56, 57 (16), 63, 77, 81
Thermoactinomyces (*Micromonospora*) *vulgaris*	20, 22, 25, 27, 40, 51, 81
T. sacchari	49, 51
Thermoactinomyces sp.	77
Thermomonospora curvata	22, 50, 71, 74 (73)
T. viridis (*Thermoactinomyces* and *Thermopolyspora glauca*)	22, 25, 43, 51
Thermomonospora sp.	43, 55, 81 (51)
Other bacteria	
Bacillus circulans? (or *B. coagulans*?)	55
B. coagulans (type B)	62
B. licheniformis	25, 40, 57
? *B. sphaericus*[c]	62
B. stearothermophilus (*B. calfactor*)	27 (67), 32, 41, 44, 53, 55, 62
B. subtilis	27 (67), 41, 55
Bacillus sp.	9, 28, 45, 48, 68, 82; Morris[e]
Clostridium thermocellum	43
Clostridium spp.	31, 80
? *Micrococcus* sp.[c]	25
? *Pseudomonas fluorescens*[c]	63
Pseudomonas sp.[c]	41, 71
Unidentified nonsporeforming coccus-rod	55

[a] Species are given with modern names, to the extent possible. Other names used by some authors, if now considered a probable synonym, are given in parentheses, as are the names of imperfect stages of fungi. Where the original author misidentified or did not identify an isolate, the citation giving the correct identification is placed in parentheses following the original reference. Additional reviews and references used in compiling the table and in determining modern species names and synonyms included references 2, 4, 6, 15, 17, 26, 29, 33, 35, 37–39, 58, 64, 70, 76, 83, and 84.

[b] A question mark preceding the name of a species indicates that none of the work cited actually demonstrated growth at 50°C or above. A question mark after a name indicates that the identification or synonym is uncertain.

[c] Not listed in *Bergey's Manual of Determinative Bacteriology* (6) as capable of growth at 50°C or above.

[d] Species incertae sedis (59) or name considered illegal (7).

[e] Morris, M.S. thesis.

Two types of nonsporeforming bacteria (Table 2) were isolated from the lower-temperature laboratory trials. Also isolated in small numbers were the fungus *Aspergillus fumigatus* and representatives of two actinomycete genera, *Thermoactinomyces* and *Streptomyces*.

DISCUSSION

Fungi and actinomycetes. The fungus *A. fumigatus* and the actinomycetes *Streptomyces* spp. and *Thermoactinomyces* spp. have been widely reported in self-heating materials (Table 1). *A. fumigatus* is of interest because of its cellulolytic activity (10) and because it is an opportunistic pathogen and aeroallergen (3, 12). Some actinomycetes, including *Thermoactinomyces* spp., have been implicated in farmer's lung disease (4, 25).

Fungi and actinomycetes in general are of importance in composting, especially during the later curing stage, but it is

TABLE 2. Summary of *Bacillus* and nonsporeforming bacterial isolates from thermophilic solid-waste composting[a]

Taxon	No. of:		Catalase	Anaerobic growth	VP		Growth at or in:					
	Samples in which present	Isolates			Reaction	pH	65°C	60°C	20°C	5% NaCl	7% NaCl	pH 5.7
B. licheniformis	7	33	+	+	+	5.2–8.4	–	3 (1)/4			+	+
B. subtilis	1	3	+	–	+	6.9–8.8	–				+	+
B. coagulans type A	3	45	+	–	–	4.4–4.9	+				–	+/40
B. coagulans type B	3	130	129+ 1–	+ +	+ +	4.1–5.2 4.4	(6) –	+/96 +			–/42 –	+ +
B. circulans complex	7	264	+	245 (13)[f]	–	4.8–6.9	–	65 (3)/78[g]	7/12	1/4	13/227	5 (1)
B. stearothermophilus	7	215	214	–	–	5.0–7.1	+				–/185	1/137
B. brevis	5	24	+	–	–	6.2–8.9	(1)[l]	+/7			–/22	6
B. sphaericus	4	24	+	–	–	7.8–8.6	–	19/20		–/19	–/19	–
Unassigned *Bacillus* sp. type i	1	8	–	–	–	6.0–6.2	–	+	–	–	–	–
Unassigned *Bacillus* sp. type ii	3	6	+	+	–	5.2–5.4[m]	–	+	2 (1)	4	–	3
Nonsporeformer type a[n]	2	42	+/22	–/22	–/22	6.2–8.4	–/22				–/22	2/22
Nonsporeformer type b[o]	3	50	+	–	–	7.9–8.3	–				(1)[h]	–

likely that bacteria predominate during the earlier thermophilic stage. The reasons for this predominance are not entirely clear, but undoubtedly involve interactions between temperature, pH, moisture content, oxygen concentration, available carbon sources, and inherent maximum rates of proliferation.

Nonsporeformers. Two types of nonsporeforming bacteria were isolated from the lower-temperature composting runs and predominated in one of them (run A). Identification of these isolates was not confirmed, but type a may have been similar to members of the genus *Pseudomonas*, and type b may have been similar to members of the genus *Arthrobacter*. Neither of these genera (nor any other genus to which these isolates might have been assigned) contains species reported capable of growth at 50°C or above (6). However, there are two reports of *Pseudomonas* sp. growing at 50 to 55°C in mushroom compost (41, 71), and a coccobacillus was observed in composting refuse-sludge mixtures at 50°C (55).

***Bacillus* species.** Numerous authors have reported the presence of *Bacillus* spp. in self-heating materials at thermophilic temperatures, often as the major component of the microbiota (Table 1). Species reported include *B. licheniformis*, *B. subtilis*, *B. coagulans* type B, *B. stearothermophilus*, and *B. sphaericus*. All of these species, in addition to several others, were also found in the present study.

A large majority of the total number of isolates were members of the genus *Bacillus* (87% of the randomly picked colonies). Most of these isolates could be readily assigned to a recognized species. However, about 15% were assigned to species only by allowing for greater variability in one or more characteristics than has been reported by other authors for their strains. In particular, growth at higher temperatures than previously reported was found for strains of several species. A small number of isolates (less than 2%) could not be assigned to any recognized species.

The imperfect match of some isolates with previously described species is not unique to the present work. For example, such imperfect matching was also true of 12 to 15% of the strains of Gordon et al. (35). Additionally, the temperature of incubation has been shown to affect the growth requirements of some *Bacillus* strains. Campbell and Williams (8) found that when eight strains of *B. coagulans* and one strain of *B. globigii* (possibly *B. licheniformis* or *B. subtilis*) were incubated at 36, 45, and 55°C, three patterns of results were seen with about equal frequency. For some strains, there were no changes in the amino acids or vitamins required for growth; for other strains, there were additional requirements at the higher temperatures; and for the rest of the strains, there were additional requirements at the lower temperatures. Farrell and Campbell (21) expressed the belief that such effects occurred among thermophiles in general, and Cross (16) likewise noted that the medium will affect the growth of thermophilic actinomycetes differently at different temperatures. It is easy to imagine the loss of particular functions, such as the ability to hydrolyze starch or deaminate phenylalanine, among strains growing at temperatures near their maximum. These factors were kept in mind in assigning to species the isolates from composting material. The work of Gordon et al. (35), which reports the results for a large number of strains individually, is used as the primary basis for comparison.

(i) *B. licheniformis*. Most of the 33 isolates of *B. licheniformis* conformed closely to the strains described by Gordon et al. (35). Although an increase in pH during incubation in Voges-Proskauer (VP) broth (as occurred with many of the composting isolates) was not noted with any of the strains used for that report, such an increase had previously been noted on occasion (67). Growth of three

TABLE 2.—*Continued*

Forms acid from:				Starch hydrolysis	Utilization of:		Nitrate reduction	Phenylalanine deamination	Decomposition of:		Litmus milk reaction[b]	Spore shape[c]	Swelling of sporangia[d]
Glucose	Arabinose	Xylose	Mannitol		Citrate	Propionate			Casein	Tyrosine			
+				+	+/31	+/26	+/31					E	S (1)
+				+	2	−	+					E	S
+				+ (7)	6 (1)/40	1/31	(2)/40[e]					E	S, A
+				+	−/33	−/1	23/33					E (1)	A, S (1)
+				+	−	−	−				ARC, 1	E	A
262 (12)[f]	12 (3)/19[h]	12 (2)/19	5 (1)/19	+ (1)	−/31	−/17	71 (2)/227		2/6		N, 8; AR, 1; ARC, 5; ADRC, 1	E (76)	A (107)[i]
+ (3)/149				206 (1)[j]	4/33	5 (1)/30	6/41					E[k]	A, S (1)
13 (5)[g]				−	6 (1)/16	−/9	10 (1)		+/7	+/7		E (2)	A (6)
−			−/3	−	−/3	−/3	−/22	(13)/16		−/2	N, 14	ES or S	A (5)
+	−/3	+/3	+/3	+	−	−	+				BDR, 3	E	A, S
+	5	3	−	−	−		3	−/4	−/5	−/4	A, 2; AR, 1; ARC, 3	E	A
(5)/22				−/22	(1)/22	−/17	−/22						
−				30[p]	4 (3)	−/46	−						

[a] Number of isolates positive for each test is given, with the number of these which were weak given in parentheses. +, All tested isolates were positive; −, all tested isolates were negative. If not all isolates were tested, the number tested is given as the denominator.
[b] Number giving each type of reaction is shown (others not tested). A, Acid; B, alkaline; C, curd; D, digestion of casein; N, neutral; R, reduction of litmus.
[c] E, Ellipsodal; S, spherical; ES, mostly ellipsoidal but some spherical.
[d] S, Slight; A, appreciable; when both occur, the more common occurrence given first. The number of isolates for which no spores or sporangia were observed is given in parentheses.
[e] Another three isolates were ±.
[f] Another two isolates were ±.
[g] Another eight isolates were ±.
[h] Another isolate was ±.
[i] For five isolates, swelling was slight (not appreciable).
[j] Another five isolates were ±.
[k] One strain also had some spherical spores.
[l] Another six isolates were ±.
[m] One strain showed pH values of 7.5 and 5.1 in two trials.
[n] Gram-negative rods, 0.3 to 0.4 by 0.8 to 1.5 μm; +/2 oxidase; colonies on TSA, translucent at 24 h and mucoid and brown at 48 h.
[o] Gram-variable cocci, coccobacilli, or rods, 0.3 to 0.6 by 0.3 to 0.8 μm; colonies on TSA, cream colored.
[p] Starch-positive isolates also appeared to liquify agar.

cultures at 60°C would appear to extend the maximum temperature for growth of this species beyond the 55°C previously reported. This species was found in small numbers in all the samples except the laboratory runs at 60°C and above.

(ii) *B. subtilis*. The three isolates of *B. subtilis*, all from the two Dano drums at Leicester, conformed closely with previous reports for the species (35).

(iii) *B. coagulans* type A. Three laboratory composting samples yielded a total of 45 isolates which were assigned to the *B. coagulans* type A group. No strains of this type were examined by Gordon et al. (35); but, based on the report by Wolf and Barker (84), personal communication with Gordon concerning later additions to her culture collection, the uniformity of the test results, and the ease of separation from other isolates, this grouping seemed appropriate. Type A strains differ from the more commonly observed type B strains mainly in their failure to produce acetoin (VP negative) and their ability to grow at 65°C. All of the strains reported here also failed to grow in anaerobic agar, a variable characteristic for the type. The negative result reported by Wolf and Barker (84) for starch hydrolysis may have been due to their use of a less sensitive method. Many of the strains isolated here initially showed a very weak or even negative reaction on starch, but on subsequent transfers, they readily hydrolyzed this substrate. There was some tendency among the type A isolates to show less swelling of the sporangium than did the type B isolates.

This group in some ways is intermediate between *B. coagulans* type B and *B. stearothermophilus* and is also somewhat similar to *B. circulans*. Isolates from hot springs and canned food, considered intermediate between *B. stearothermophilus* and *B. coagulans* (52), appear similar to isolates of *B. coagulans* type A as described here. The problem of intermediates in reference to these species has been discussed (30).

(iv) *B. coagulans* type B. A small number of the 130 isolates from three samples that were assigned to the *B. coagulans* type B group yielded a pH in VP broth of 4.9 to 5.2 (rather than 4.8 or below), and one strain was repeatedly catalase negative. Otherwise, all isolates fit the typical pattern (35) for *B. coagulans*.

(v) *B. circulans* complex. All samples except those from the laboratory composting runs at temperatures of 60°C and

above yielded isolates which were assigned to the *B. circulans* complex, for a total of 264 isolates. While many of these isolates fit the description of this species quite well, others did not. This problem was especially troublesome because of the already high degree of heterogeneity in this group.

Various additional methods have been tried by others in an attempt to better separate *B. circulans* from other species or to divide it into more homogeneous subunits. For example, Proom and Knight (60) studied the minimal nutritional requirements of seven *Bacillus* taxa and found that in contrast to the other groups, *B. circulans* was very heterogeneous in this regard as well. Using electron microscopy, Bradley and Franklin (5) surveyed the surface configuration of spores in 62 strains of *Bacillus* spp. representing 19 species and 4 varieties. *B. circulans* and *B. alvei* were found to have intermediates in this characteristic, as well as in their biochemical test results. Using paper chromatography, Jayne-Williams and Cheeseman (46) found that strains of most *Bacillus* species gave fairly uniform results, but *B. circulans* strains did not. In view of these results, the use of the term complex, rather than species, seems appropriate.

The composting isolates assigned to this complex were also highly heterogeneous. The tested isolates from Altoona failed to form acid from the carbohydrates tested other than glucose. This failure would suggest *B. alvei*, or the related but VP-negative *B. thiaminolyticus*, as an alternative identification, except that the characteristic side-by-side arrangement of the spores in these two species was not observed.

Most of the *B. circulans* composting isolates were able to grow in anaerobic agar. A few isolates which could not were similar to the *B. firmus-B. lentus* series (35, 36) in that they showed only a slight swelling of the sporangium and a pH in VP broth of 6.2 to 6.4. While five such cultures were unlike *B. firmus-B. lentus* in that they were unable to grow in 7% NaCl (or even 5% NaCl for three isolates), three cultures were capable of such growth. (Sporangia were not observed or were seen rarely for several of these strains.) None of these three cultures could form acid from mannitol, however, and each appeared closely related to other isolates from the same samples which were more clearly assignable to *B. circulans*.

An important difference between many of the composting isolates and the *B. circulans* strains studied by Gordon et al. (35) was the ability to grow at thermophilic temperatures. Whereas only 6 of the 38 strains studied by those authors were capable of growth at 50°C, all of the composting isolates could grow at this temperature, and many of them were capable of growth at 60°C. Previously, only one isolate of *B. circulans* capable of growth at 55°C and none that could grow at higher temperatures were reported (34). Growth at 60°C tends to further blur the boundaries of this already amorphous complex. Differentiation between certain *B. circulans* and *B. stearothermophilus* isolates is then solely based on a 5°C difference in maximum growth temperature. No strains of *B. alvei*, *B. thiaminolyticus*, *B. firmus*, or *B. lentus* capable of growth at 50°C or above have been reported (35, 36).

Previous reports of thermophilic *B. circulans* strains do exist but are not definitive. Egorova (19), for example, investigated thermophilic growth rates and yields using (among other species) five strains of obligately thermophilic *B. circulans*. The basis on which these strains were differentiated from *B. stearothermophilus* is not clear, however. A review by Farrell and Campbell (21) also reported the isolation of thermophilic strains of *B. circulans*, citing unpublished data by one of the authors. Allen (1) recognized that her thermophilic strains resembling *B. circulans* were properly identified as *B. stearothermophilus*, and it is possible that this observation should apply to the strains of Campbell and Egorova as well. Tischer et al. (78) tentatively identified their obligately thermophilic isolate from sewage oxidation ponds as *B. circulans*, but it is probable from their description that they were dealing with *B. stearothermophilus*. The reported failure of their strain to grow at 65°C or produce acid from glucose may have been the result of the medium used. (A broth was used for the high-temperature growth tests, which made inadequate aeration probable.) The possibility that the thermophilic isolates from composting reported here were similarly *B. stearothermophilus* is unlikely because of the ability of most of them to grow in anaerobic agar.

A fairly large number of isolates from Leicester and one from the completely mixed composting laboratory simulation failed to sporulate or sporulated only very poorly on the laboratory media provided (soil extract agar and, additionally for some strains, BBL AK sporulation agar). These isolates were classified as members of the genus *Bacillus* on the basis of their similarity to sporeforming strains from the same samples and their lack of similarity to any other recognized genus. A possible selective advantage of poor sporulation in continuously thermophilic composting exists, in that cells which sporulate are likely to be permanently removed from the reactor before they germinate. Failure to sporulate, on the other hand, results in no disadvantage provided reactor conditions are maintained in a favorable range.

(vi) *B. stearothermophilus*. *B. stearothermophilus* was obtained from every composting sample except those from the Dano drums and the lower-temperature windrow at Leicester, for a total of 215 isolates. The higher-temperature laboratory composting samples, in fact, appeared to be nearly monocultures of *B. stearothermophilus*.

For the most part, the composting isolates demonstrated the typical properties for this species. Four isolates failed to hydrolyze starch, and another five did so only very weakly, but others have reported similar results with some strains (35, 52).

A small number of the isolates assigned to this species produced only a slight swelling of the sporangium during sporulation. This characteristic was also noted by Wolf and Barker (84), who suggested splitting the species into three groups based on this and other characteristics.

(vii) *B. brevis*. A total of 24 isolates from five samples was assigned to *B. brevis*. Over a third of these isolates produced a pH of 6.2 to 6.6 in VP broth, rather than the 8.0 or above expected. A small number failed to form acid from glucose, and others produced very little, but a negative reaction for this test was previously reported for 3 of 21 strains (35). Growth of *B. brevis* at 60°C has been reported previously, but a small number of these isolates from composting appeared able to grow very weakly even at 65°C.

(viii) *B. sphaericus*. Twenty-four isolates from four samples were assigned to *B. sphaericus*, although only after considerable hesitation. None of the 29 strains examined by Gordon et al. (35) was capable of growth at 50°C or higher, and the maximum temperature for growth given in *Bergey's Manual of Determinative Bacteriology* is 45°C (30). This species has therefore previously been considered strictly mesophilic. Furthermore, many of the composting isolates produced ellipsoidal as well as spherical spores.

Differentiation between *B. sphaericus* and *B. brevis* on the

basis of biochemical tests can be difficult. A positive glucose, mannitol, or tyrosine test would be diagnostic for *B. brevis*, but several strains of that species are negative for one or more of these tests (35). A positive nitrate reduction test would also be strong evidence of *B. brevis*, although one *B. sphaericus* strain was also positive (35). On the other hand, growth in 5% NaCl or deamination of phenylalanine would identify a strain as *B. sphaericus*, although not all strains are positive for these tests (35). It is clear that negative test results are of little help in distinguishing the two species, since only positive results are unique to one or the other. Results with the earlier isolates were negative for all six tests, but the isolates from Leicester yielded a number of weakly positive reactions for phenylalanine deamination. It was felt that these results made assignment to *B. brevis* unsatisfactory, and since no other species seemed even remotely appropriate, the isolates were classified as *B. sphaericus*.

Other reports of thermophilic *B. sphaericus* strains may be found in the literature. Allen (1) reported the isolation of strains capable of growth at 65 to 70°C whose "characteristics, with the exception of temperature range, are closely similar to those of *B. sphaericus*." Three strains isolated by Hollaus from sugar beets were identified in 1985 by Gordon as thermophilic *B. sphaericus* (R. E. Gordon, personal communication). Rothbaum (62) picked a colony from a plate incubated at 60°C (used in counting thermophiles in self-heating wool) which was later identified as *B. sphaericus*. Its maximum growth temperature in pure culture was only 47.5°C, however, and he therefore dismissed it as a contaminant.

Another report of a *B. sphaericus* culture growing at thermophilic temperatures—and a possible explanation of the results of Rothbaum (62)—comes from the work of Sie et al. (65). These authors felt that they had obtained a factor from *B. stearothermophilus* which, when added to a culture of mesophilic *B. sphaericus*, allowed the latter to grow at 55°C (even after continuous subculturing without added factor). Yeast autolysate also enabled the mesophilic *B. sphaericus* to grow at 55°C, but only if the yeast autolysate was added to the subculturing medium. Perhaps, rather than the thermophilic "factor" which they felt they had demonstrated, they really had shown an additional requirement for some growth factor at thermophilic temperatures. As already noted, Campbell and Williams (8) have shown a requirement for additional amino acids or vitamins or both in some *Bacillus* strains at high temperatures.

(ix) Unassigned strains, type i. One low-temperature laboratory composting run yielded eight isolates which could not be placed in any recognized *Bacillus* species. The negative catalase reaction and other results were suggestive of *B. stearothermophilus*, but none of the eight could grow at 65°C. (Attempts to obtain growth at 65°C with two other soil extract media formulations, one including yeast extract, were also unsuccessful.) No catalase-negative strains of *B. circulans* have been reported, precluding grouping with that complex. These strains thus appear to be intermediate between those two species.

(x) Unassigned strains, type ii. Six isolates from three samples appeared to differ significantly from any previously described *Bacillus* strains. Their ellipsoidal spores, which appreciably swelled the sporangium, would place them in group II of Gordon et al. (35). Failure to hydrolyze starch, coupled with ability to grow in anaerobic agar, indicates a possible relationship to *B. laterosporus*, but the composting isolates differed from that species in a number of tests. Furthermore, microscopic observation did not reveal the distinctive C-shaped parasporal body from which the species derives its name. Agreement with the somewhat similar *B. pulvifaciens* is equally poor. Assignment to *B. stearothermophilus* as a starch-negative variant is inappropriate because of the ability to grow in anaerobic agar, failure to grow at 65°C, and production of acid in litmus milk. Placement in *B. circulans* as a thermophilic, starch-negative variant would further blur the boundaries of this complex, especially in view of the failure of all strains to produce acid from mannitol. No relationship to any of the unassigned strains of Gordon et al. (35) or to any of the poorly represented strains of *Bergey's Manual of Determinative Bacteriology* (30) is apparent. Designation of a new species may be justified in the future.

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